13. (Amended) A method of measuring the level of an isoprostane molecular marker for lipid peroxidation in a mammal suspected of having an oxidant stress syndrome or disease, said method comprising

a) obtaining a sample of a tissue or body fluid from said mammal;

b) isolating from said sample said isoprostane molecular marker by using a total lipids solvent extraction method; and

c) quantifying the level of said isoprostane molecular marker.

Please cancel claims 6 and 7 without prejudice to the inclusion of the subject matter contained therein in any later filed continuation or divisional application.

REMARKS

The present invention relates to compositions and methods useful in the assessment of the level of lipid peroxidation in a mammal.

Claims 1-34 are pending in the application.

Claims 1, 11, and 13 have been amended to more particularly and distinctly claim the subject matter which the Applicants regard as their invention. Claim 6, which depended from claim 1, has been canceled without prejudice herein and the substance of claim 6 has been incorporated into both claims 1 and 11. Claim 7, which depended from claim 1, has been canceled without prejudice herein and the substance of claim 7 has been incorporated into both claims 1 and 11. Claims 1 and 11 have been amended herein to recite that the suspected oxidant stress syndrome or disease is Alzheimer's disease and to recite that the isoprostane molecular marker for lipid peroxidation is selected from the group consisting of $iPF_{2\alpha}$-III, $iPF_{2\alpha}$-VI, and 8,12-*iso*-iPF_2-VI. Claim 13 has been amended herein to clarify what is intended, i.e., isolating a marker followed by quantifying the amount of the marker. Support for these amendments is found throughout the specification as filed and as more fully set forth below. Thus, no new subject matter has been added by way of these amendments.

Applicants note that an Information Disclosure Statement (IDS) with an accompanying Form 1449 was filed February 1, 2002. However, an initialized copy of the

Form 1449 has not been returned by the Examiner. A copy of the IDS and Form 1449 is enclosed herein for the Examiner's convenience and Applicants respectfully request that the Examiner return an initialized copy of the Form 1449 to Applicants at his earliest convenience.

Rejection of Claims 1-23 pursuant to 35 U.S.C. § 102(a or b)

Claims 1-23 stand rejected pursuant to 35 U.S.C. § 102(a or b) as being anticipated by Pratico et al., (1998, FASEB J. 12:15:1777-1783). In the Examiner's view, Pratico teaches that iPF2a-III and iPF2a-VI, measured by GC/MS, are elevated in Alzheimer's Disease. It is also the view of the Examiner that Pratico teaches all the features in the claims for the same function as claimed.

Preliminarily, claims 6 and 7 have been cancelled herein and Applicants submit that the rejection is now moot as to these claims.

Further, Applicants assert that Pratico cannot form the basis for a 102(a) rejection or a 102(b) rejection as prior art, for the reasons set forth below. First, as stated on page 2 of the Office Action, the present application is entitled to priority pursuant to 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 60/110,569, which was filed on December 2, 1998. The particular issue of FASEB Journal in which Pratico was published was mailed from the publisher on December 4, 1998, two days after the priority date to which this application is entitled. Thus, Applicants respectfully submit that Pratico does not anticipate the present application under 35 § U.S.C. 102(a or b) because it is not prior art, and Applicants therefore request that this rejection be reconsidered and withdrawn.

Rejection of Claims 1-5 and 10-12 pursuant to 35 U.S.C. § 102

Claims 1-5 and 10-12 stand rejected pursuant to 35 U.S.C. § 102 as being anticipated by each of Morrow (U.S. Pat. No. 5,891,622), Roberts (5,700,654), Mardini (Circulation, 1998, 98:17:17), Rokach (Rokach et al., 1998, Recent Res. Devel. in Organic Chem. 2:393-407), Pratico (Pratico et al., 1997, Prostaglandins and Control of Vascular Smooth Muscle Cell Proliferation 48:25-41), FitzGerald (FitzGerald, 1996, FASEB J. 10:6:A1138), Maxey (Int. Publication No. WO 94/04921) and Reilly (Reilly et al., 1998,

Circulation 98:25:2822-2828). Applicants respectfully submit that none of the aforementioned references anticipate claims 1-5 and 10-12 of the present invention under 35 U.S.C. § 102.

Although the Examiner, at page 2, lines 1-3 of the Office Action, lists Morrow as one of the references asserted to anticipate claims 1-5 and 10-12 under 35 U.S.C. § 102(a or b), the Examiner cited a 102(e) date of 2/1997 for Morrow at line 5. Therefore, Applicants presume that the Examiner has rejected Morrow under 102(e) and respond accordingly. The Examiner contends that Morrow teaches that isoprostane concentrations correlate with free radical production and this measurement has diagnostic potential (see column 1, last paragraph). Applicants respectfully submit that Morrow cannot anticipate claims 1-5 and 10-12 under 35 U.S.C. § 102(e), as more fully set forth below. Preliminarily, it is well-settled law that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." MPEP §2131 (quoting *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)). "The identical invention must be shown in as complete detail as is contained in the . . . claim." *Id.* (quoting *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989) (emphasis added). Therefore, Morrow must describe each and every element of each of claims 1-5 and 10-12 in order to anticipate these claims under Section 102, and Applicants respectfully submit that this reference does not. Morrow merely teaches a method of assessing the existence of oxidative stress by measuring isoprostanes (see column 2, lines 43-61 and claims 1-10). Morrow does not teach a method of measuring the level of lipid peroxidation in a mammal wherein increased levels of lipid peroxidation associated with an oxidant stress syndrome or disease comprise an elevated level of a reactive oxygen species or an elevated level of inflammation, further wherein the elevated level of inflammation comprises elevated cyclooxygenase (COX) activity, all of which are claimed in the present invention (see claims 1-5 and 10-12, and Examples 1 and 2).

Furthermore, Morrow teaches the use of a single isoprostane, namely 8-epi-prostaglandin F2α (8EPGF2) (see column 1, lines 31-42; Example 1, columns 9 and 10, Example 2), not the use of the isoprostane markers 8,12-*iso*-iPF$_2$-VI, iPF$_{2\alpha}$-III (formerly

known as 8-*iso*-PGF_2), and $iPF_{2\alpha}$-VI. Moreover, Morrow teaches a method of separating the glucuronidated form from an unglucuronidated form of an isoprostane in a sample, a method not claimed in the present invention. Furthermore, Morrow does not teach isolating an entire species of an isoprostane, nor does it teach or claim using such as a diagnostic. In addition, Morrow teaches measuring oxidant stress in the diseases or disorders such as hepatorenal syndrome, atherosclerosis, and carcinogenesis (column 1, lines 60-65), but not in Alzheimer's disease.

Although not necessarily agreeing with the view of the Examiner, Applicants, in a good faith effort to expedite prosecution of the application, have amended claims 1 and 11 to recite diagnosis of only Alzheimer's disease. This amendment does not introduce new subject matter and is fully supported throughout the specification as filed (see Figures 1-7; Tables 2-5; pages 10-40; claim 6, canceled herein). Applicants respectfully submit that this amendment overcomes the rejection of claims 1 and 11 and their dependent claims because Morrow does not teach diagnosing Alzheimer's disease by measuring isoprostane markers.

The present invention claims a method for measuring isoprostane molecular markers for lipid peroxidation in a mammal suspected of having Alzheimer's disease, further wherein the isoprostane molecular markers are selected from the group consisting of 8,12-*iso*-iPF_2-VI, $iPF_{2\alpha}$-III, and $iPF_{2\alpha}$-VI (amended claims 1 and 11 and their dependent claims), a method of measuring lipid peroxidation in cerebrospinal fluid (claim 10) and a method for diagnosing Alzheimer's disease in a mammal (amended claim 11 and claim 12). The amendments to claims 1 and 11 introduce no new subject matter and are fully supported throughout the specification as filed (see Figures 1-7; Tables 2-5; pages 10-40; claims 6 and 7, canceled herein). Morrow does not teach, nor does it mention, that elevated levels of lipid peroxidation may comprise elevated levels of a reactive oxygen species, or that elevated levels of inflammation comprise elevated cyclooxygenase activity. Also, Morrow does not teach the use of a biological sample such as cerebrospinal fluid to measure isoprostane levels, nor does it teach the measuring the levels of the isoprostane markers selected from the group consisting of 8,12-*iso*-iPF_2-VI, $iPF_{2\alpha}$-III, and $iPF_{2\alpha}$-VI. Therefore, Morrow does not teach each and every element of these claims.

Applicants respectfully submit that Morrow does not anticipate the present invention and Applicants respectfully request that the rejection of claims 1-5 and 10-12 under 35 U.S.C. § 102(e) should be reconsidered and withdrawn.

Although the Examiner, at page 2, lines 1-3 of the Office Action, lists Roberts as one of the references asserted to anticipate claims 1-5 and 10-12 under 35 U.S.C. § 102(a or b), the Examiner cited a 102(e) date of 6/1991 for Roberts at line 16. Therefore, Applicants presume that the Examiner has based the anticipation rejection under § 102(e) and respond accordingly. The Examiner asserts that Roberts teaches measuring oxidative stress by measuring the amount of non-cyclooxygenase derived metabolites of prostanoids in a sample (see column 3), that prostaglandin F2-like compounds may participate in oxidative stress and may be useful in disease treatment (see column 5, lines 15-20), that assessing oxidant status in tissue by measuring Prostaglandin F2-like compounds esterified to tissue phospholipids could be used to obtain evidence of free radical induced injury (column 7), and that prostaglandin F2-like compounds or their metabolites can be measured by mass spectroscopy analysis, and that the assay of Roberts can be used to measure a variety of biological fluids. Applicants respectfully submit that Roberts cannot anticipate claims 1-5 and 10-12 under 35 U.S.C. § 102(e), as more fully set forth below.

Roberts must describe each and every element of each of claims 1-5 and 10-12 in order to anticipate these claims under Section 102(e), and Applicants respectfully submit that Roberts does not.

As asserted by the Examiner, Roberts teaches a method of assessing oxidative stress by measuring the amount of noncyclooxygenase derived prostanoids in a sample. The present invention, instead, claims measuring isoprostane molecular markers for lipid peroxidation in Alzheimer's disease, further wherein the isoprostane molecular markers are selected from the group consisting of 8,12-*iso*-iPF$_2$-VI, iPF$_{2\alpha}$-III, and iPF$_{2\alpha}$-VI (see amended claims 1 and 11, as amended herein, and their dependent claims). As noted by the Examiner, Roberts teaches restricting the marker to only those which are noncyclooxygenase derived. Furthermore, Roberts does not teach the use of the isoprostane markers 8,12-*iso*-iPF$_2$-VI, iPF$_{2\alpha}$-III, and iPF$_{2\alpha}$-VI. In addition, Roberts does

not teach measuring the level of lipid peroxidation in a mammal suspected of having an oxidant stress syndrome, wherein such syndrome is Alzheimer's disease, by comparing the level of an isoprostane of an molecular marker for lipid peroxidation to that in a non-diseased animal, further wherein the marker is purified before measurement, and further wherein the lipid peroxidation comprises an elevated level of a reactive oxygen species. Roberts also does not teach, nor does it mention, that the elevated level of lipid peroxidation may comprise elevated inflammation, further wherein the elevated level of inflammation may comprise elevated cyclooxygenase activity. Although not necessarily agreeing with the view of the Examiner, Applicants, in a good faith effort to expedite prosecution of the application, have amended claims 1 and 11 to recite measuring the level of lipid peroxidation wherein the oxidant stress syndrome or disease is Alzheimer's disease. Roberts does not teach measuring lipid peroxidation in Alzheimer's disease, therefore the amendments to claim 1 and claim 11 (see above) to recite Alzheimer's disease and to recite the use of the isoprostane markers 8,12-*iso*-iPF_2-VI, $iPF_{2\alpha}$-III, and $iPF_{2\alpha}$-VI to overcome the rejection of claims 1-5 and 10-12. Therefore, Applicants respectfully submit that Roberts does not anticipate claims 1-5 and 10-12 under 35 U.S.C. § 102(e) and respectfully request that the rejection be reconsidered and withdrawn.

Next, at page 4 of the Office Action, the Examiner contends that Mardini teaches four families of isomers of prostaglandin F2 are effective markers of oxidative stress and pathologic conditions. Applicants respectfully submit that Mardini does not anticipate claims 1-5 and 10-12 under 35 U.S.C. § 102(a or b), for the following reasons. Contrary to the assertion of the Examiner that Mardini teaches that isomers of Prostaglandin F2 are effective markers of pathologic conditions, Applicants assert that this reference merely speculates that the isomers "may play a role in some pathologic conditions" (see lines 3-4 of abstract), and does not state or assert that they are markers of pathologic conditions. Furthermore, Mardini only teaches an assay in a human model of limited general inflammation, not other oxidant diseases such as Alzheimer's Disease. Mardini does not teach, nor does it mention, a method of measuring the level of lipid peroxidation in a mammal wherein increased levels of lipid peroxidation associated with an oxidant stress syndrome or disease comprise an elevated level of a reactive oxygen

species or an elevated level of inflammation, further wherein the elevated level of inflammation comprises elevated cyclooxygenase activity.

Therefore, Applicants respectfully submit that Mardini does not anticipate claims 1-5 and 10-12 under 35 U.S.C. § 102(a or b) and respectfully request that the rejection be reconsidered and withdrawn.

In addition, claims 1-5 and 10-12 stand rejected pursuant to 35 U.S.C. § 102(a or b) as being anticipated by Rokach. Applicants respectfully submit that Rokach does not anticipate claims 1-5 and 10-12. In the Examiner's view, Rokach teaches that a significant development in the field has been the identification of iPF2a-I in human urine using a synthetic standard and its deuterated derivative (see page 405). However, Rokach made no mention of, nor does it teach, the use of isoprostane markers 8,12-*iso*-iPF_2-VI, $iPF_{2\alpha}$-III, and $iPF_{2\alpha}$-VI. In addition, based on the teachings of Rokach, one skilled in the art would not have appreciated the use of a method of measuring the level of lipid peroxidation in a mammal wherein increased levels of lipid peroxidation associated with an oxidant stress syndrome or disease comprise an elevated level of a reactive oxygen species or an elevated level of inflammation, further wherein the elevated level of inflammation comprises elevated cyclooxygenase activity. Further, Rokach made no mention of, nor does it teach or contemplate, measuring isoprostanes in various samples such as cerebrospinal fluid.

Therefore, Applicants respectfully submit that Rokach does not anticipate claims 1-5 and 10-12 under 35 U.S.C. § 102(a or b) and respectfully request that the rejection be reconsidered and withdrawn.

The Examiner also asserts that the review article by Pratico (Pratico et al., 1997, Prostaglandins and Control of Vascular Smooth Muscle Cell Proliferation 48:25-41) teaches that the F2 series and 8-epi PGF2a are potential indices of oxidant stress in vivo (see page 27). Therefore, Pratico must describe each and every element of each of claims 1-5 and 10-12 in order to anticipate these claims under Section 102(a) or 102(b), and Applicants respectfully submit that Pratico does not describe each and every element of claims 1-5 and 10-12.

Based on the teachings of Pratico, one of skill in the art would not have appreciated a method of measuring the level of lipid peroxidation in a mammal with Alzheimer's disease, wherein an elevated level of lipid peroxidation indicates the presence of an oxidant stress syndrome or disease in the animal, isolating an isoprostane molecular marker from a sample prior to its measurement, that an elevated level of lipid peroxidation may comprise an elevated level of a reactive oxygen species, that an elevated level of lipid peroxidation as measured by the method of claim 1 may comprise an elevated level of inflammation, further wherein the elevated level of inflammation comprises elevated cyclooxygenase activity. Furthermore, Pratico does not teach, nor does it mention, the use of the isoprostane markers 8,12-*iso*-iPF_2-VI, iP$F_{2\alpha}$-III, and iP$F_{2\alpha}$-VI.

Therefore, Applicants respectfully submit that Pratico does not anticipate each and every element as set forth in claims 1-5 and 10-12, and request that the rejection be reconsidered and withdrawn.

Claims 1-5 and 10-12 stand rejected under 35 U.S.C. § 102(a or b) as being anticipated by FitzGerald. It is the Examiner's opinion that FitzGerald teaches in the abstract that 8-epi-PGF2a is an index of oxidant stress. Applicants respectfully submit that FitzGerald does not teach a method of measuring the level of lipid peroxidation in a mammal wherein an elevated level of lipid peroxidation indicates the presence of an oxidant stress syndrome or disease in the animal wherein the oxidant stress syndrome or disease is Alzheimer's disease. In addition, based on the teachings of FitzGerald, one of skill in the art would not appreciate isolating an isoprostane molecular marker from a sample prior to its measurement, wherein said elevated level of lipid peroxidation comprises an elevated level of a reactive oxygen species, or wherein, the elevated level of lipid peroxidation comprises an elevated level of inflammation, further wherein the elevated level of inflammation comprises elevated cyclooxygenase activity. Indeed, FitzGerald is silent with respect to these methods.

Furthermore, FitzGerald does not teach the isoprostanes of the present invention as outlined above in the Morrow and Roberts discussions, nor does it teach the use of biological samples such as cerebrospinal fluid or plasma for measuring isoprostane levels. In addition, Fitzgerald, does not set forth a method of diagnosing an oxidant stress

syndrome or disease by obtaining a sample from a test mammal and comparing the sample to a second sample from an otherwise identical animal which is not afflicted with the oxidant stress syndrome or disease, nor does it teach isolating the isoprostane marker from the first sample after obtaining the sample, but before assessing the level of marker present. Applicants assert that FitzGerald does not anticipate claims 1-5 and 10-12 because it does not anticipate each and every element set forth in the claims.

In the view of the Examiner, Maxey teaches measuring isoprostanes in biological samples. The Examiner further asserts that a number of medical uses are discussed and that a number of isoprostanes are shown in Maxey. Applicants respectfully submit that Maxey does not anticipate claims 1-5 and 10-12 of the present invention for the following reasons. Maxey teaches away from the present invention because it teaches methods directed to isoprostane-protein conjugates, enzyme-immunoassay techniques, and methods useful for making antibodies (pages 2 and 3). In addition, Maxey teaches away from the present invention because it teaches that GC/MS is unsuited for a viable medical diagnostic test (page 2, lines 15-18) and instead teaches the use of enzyme immunoassay techniques to measure isoprostane-protein conjugates (page 7, lines 22-28). Furthermore, Maxey teaches use of the isoprostanes 8-isoprostane $F_2\alpha$, 8-isoprostane E_2, 8-isothromboxane B_2, and 9β,11β-8-isoprostane F_2 (page 3, last paragraph), but does not teach the use of isoprostane markers 8,12-*iso*-iPF_2-VI, $iPF_{2\alpha}$-III, and $iPF_{2\alpha}$-VI.

Applicants respectfully submit that, based on the teachings of Maxey, one of skill in the art would not appreciate a method of measuring the level of lipid peroxidation wherein an elevated level of lipid peroxidation indicates the presence of an oxidant stress syndrome or disease, isolating an isoprostane molecular marker from a sample prior to its measurement, use of isoprostane markers 8,12-*iso*-iPF_2-VI, $iPF_{2\alpha}$-III, and $iPF_{2\alpha}$-VI. In addition, Maxey did not teach that an elevated level of lipid peroxidation may comprise an elevated level of a reactive oxygen species, wherein, the elevated level of lipid peroxidation comprises an elevated level of inflammation, further wherein the elevated level of inflammation comprises elevated cyclooxygenase activity.

Thus, Applicants assert that Maxey does not anticipate claims 1-5 and 10-12, because it does not anticipate each and every element set forth in the claims.

The Examiner contends that Reilly teaches assays for specific isoprostanes including iPF2a-III and iPF2a-VI (noting that iPF2a-III is the same as 8-iso-PGF2a). Applicants respectfully submit that Reilly does not anticipate the present invention because it does not teach each and every element of the claims. Reilly teaches assays for measuring isoprostanes in the urine of patients with hypercholesterolemia (abstract). Reilly does not teach using urine from patients with Alzheimer's disease. Reilly does not teach measuring isoprostanes in cerebrospinal fluid, plasma, and tissues. In addition, based on the teachings of Reilly, one of skill in the art would not appreciate that the methods of measuring the level of lipid peroxidation wherein an elevated level of lipid peroxidation indicates the presence of an oxidant stress syndrome or disease, or wherein said elevated level of lipid peroxidation may comprise an elevated level of a reactive oxygen species, wherein, the elevated level of lipid peroxidation comprises an elevated level of inflammation, further wherein the elevated level of inflammation comprises elevated cyclooxygenase activity. Thus, Applicants respectfully submit that Reilly cannot anticipate the present invention because it does not teach each and every element of claims 1-5 and 10-12.

It is the view of the Examiner that all the features of claims 1-5 and 10-12 are taught by each of the above references for the same function as claimed. Applicants respectfully submit that, as described above, none of the above-identified references anticipate claims 1-5 and 10-12 of the present invention under 35 U.S.C. § 102(a, b, or e) and request that the rejections be reconsidered and withdrawn.

Rejection of Claims 24-34 pursuant to 35 U.S.C. § 103(a)

Claims 24-34 stand rejected under 35 U.S.C. § 103(a) as being, in the Examiner's view, obvious and unpatentable over Pratico (FASEB J.). The Examiner contends that Pratico teaches in the abstract, that both iPF2a-III and iPF2a-VI, measured by GC/MS, are elevated in Alzheimer's Disease. The Examiner also states that claims 24-32 differ from Pratico in that they are directed to treating disorders. It is also the Examiner's view that it would have been obvious at the time the invention was made to treat disorders diagnosed by determining isoprostanes because if the treatment were

successful, one would expect the disease marker to change. Applicants respectfully traverse this rejection. As discussed above, Pratico does not qualify as prior art because it published after the priority date of the present application. Therefore, Pratico cannot render claims 24-34 *prima facie* obvious under 35 U.S.C. § (103a). Thus, Applicants respectfully request that the rejection be reconsidered and withdrawn.

Rejection of Claims 24-34 pursuant to 35 U.S.C. § 112, first paragraph

Claims 24-34 stand rejected under 35 U.S.C. 112, first paragraph, for lack of enablement. It is the opinion of the Examiner that the claims are directed to identifying compounds for treating Alzheimer's Disease and their posology, treating disorders that raise the level of isoprostane markers, and kits, and that the specification does not enable the claims.

Applicants respectfully traverse the rejection of claims 24-34, under 35 U.S.C. § 112, first paragraph, for the reasons set forth below.

It is well-settled that an applicant need not have actually reduced the invention to practice prior to filing in order to satisfy the enablement requirement under 35 U.S.C. §112, first paragraph. MPEP §2164.02 (citing *Gould v. Quigg*, 822 F.2d 1074 (Fed. Cir. 1987)). Indeed, the invention need not contain a single example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation (*In re Borkowski*, 422 F.2d at 908), and "representative samples are not required by the statute and are not an end in themselves" (*In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970)). Thus, 35 U.S.C. § 112, first paragraph, enablement does not require any working examples.

The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. MPEP §2164.01 (citing *In re Angstadt*, 537 F.2d 498, 504 (C.C.P.A. 1976)). The fact that experimentation may be complex does not necessarily make it undue if the art typically engages in such experimentation. *Id.* Further, the specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled in the art and is already available to the public. MPEP §2164.05(a) (citing *In re Buchner*, 929

F.2d 660, 661 (Fed. Cir. 1991)). Therefore, under current law, enablement does not require a working example and experimentation is allowed, so long as it is not undue.

Under the present patent law, claims 24-34 are amply enabled by the specification as filed under 35 U.S.C. § 112, first paragraph. More specifically, claims 24-34 recite methods of identifying an effective amount of a compound useful for the treatment of Alzheimer's disease, methods of determining the optimal concentration and frequency for using a compound to treat Alzheimer's disease, and for kits (see pages 10-40). The specification as filed amply supports these claims because the skilled artisan, armed with the methods and the markers disclosed in the specification, would have been able to identify, through routine experimentation, compounds having the disclosed biological and biochemical activities as recited by the claims, and to practice the invention commensurate with the scope of the claims without undue experimentation.

Further, one of skill in the art would also be able to identify a variety of compounds and methods of using the compounds for treating Alzheimer's disease, including identifying compounds which inhibit the activity of cyclooxygenase enzyme in the brain and which reduce the level of a reactive oxygen species, following the teachings, methods, and markers, set forth in the specification as filed and/or known in the art based upon the disclosure provided in the specification without undue experimentation. That is, the crucial novel teachings of the invention, *inter alia*, the use of three markers of lipid peroxidation (page 10; page 11, lines 21-24), purifying a marker from a sample before measuring it, the use of tissue samples other than brain samples, including samples not typically affected by an oxidant stress syndrome or disease (page 16, line 28 to page 17, line 2), and methods of identifying compounds useful in treating Alzheimer's (page 19, line 21 to page 20, line 8) are amply disclosed in the specification as filed. For example, the present application discloses use of the antioxidant compounds vitamin E and vitamin C, including the frequency and doses to be used in treating Alzheimer's disease (page 19, line 21 to page 20, line 8). The specification as filed also discloses use of anti-inflammatory compounds, including non-steroidal anti-inflammatory drugs such as ibuprofen, aspirin, and cyclooxygenase-2 inhibitors, as well as their dosage and frequency of administration (page 20, lines 3-8), for treating Alzheimer's disease.

Therefore, the application merely omits that which is well-known to those skilled in the art and is already available to the public, i.e., other compounds which can be tested. Moreover, methods and assays for identifying the use and biological properties of the compounds are disclosed in the specification as filed, and/or such methods are known to those skilled in the art and the practice of such methods is routine in the art and should not be considered an undue burden (see, for example, page 3, line 20 to page 4, line 29; Figures 1-7, Tables 1-4, and Examples 1 and 2), since they were routinely performed by one skilled in the art. Thus, while the experimentation may be complex, it is certainly not undue where, as here, the art typically engaged in such experimentation at the time the specification was filed, and where there had been extensive reduction to practice where none is required.

Applicants have disclosed sufficient data to support claims reciting methods for identifying and using compounds effective for treating Alzheimer's disease or other treatments for isoprostane elevating disorders, and for kits (see Figures 1-7 and Examples 1 and 2). The specification as filed also discloses multiple assays and techniques for various types of samples to be assayed, the markers to be assayed, and methods of identifying and using compounds for the treatment of Alzheimer's disease. Thus, the skilled artisan would be able, without undue experimentation, to determine whether a compound of interest possesses the requisite activity.

Further, it is clear that the level of skill in the art would allow an artisan to easily assay numerous compounds based on the compounds disclosed in the specification as filed, in order to identify candidate compounds having the desired properties as defined, exemplified, and disclosed by the Applicants. One skilled in the art of screening compounds for those possessing a desired biological activity typically engaged in this type of experimentation at the time the specification was filed (see pages 19 and 20). This is important because the present case law regarding enablement under 35 U.S.C. § 112, first paragraph, allows significant experimentation without finding it undue if the art typically engages in such experimentation.

In the landmark enablement case of in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988), the court discussed the adequacy of disclosure with regard to a patent

disclosing an immunoassay method for the detection of hepatitis B antigen using monoclonal antibodies. The *Wands* Court noted that of 143 hybridomas produced, only nine were assayed and, of those, only four hybridomas secreted IgM antibodies and exhibited a binding affinity constant for the HBsAg determinants of at least 10^9 M^{-1}, a "respectable 44 percent rate of success." *In re Wands*, 8 USPQ2d at 1406. Finding the claims were enabled, the *Wands* Court stated:

> Wands' disclosure provides considerable direction and guidance on how to practice their invention and presents working examples. There was a high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known.
>
> The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody. No evidence was presented by either party on how many hybridomas would be viewed by those in the art as requiring undue experimentation to screen.

In re Wands, 8 USPQ2d at 1406 (emphasis added). Therefore, where, as here, the art typically screens compounds for biological activity and/or properties, e.g., capable of treating Alzheimer's disease, where compounds and their uses and markers for measuring the activity of the compounds are disclosed in the specification as filed, where the specification discloses specific compounds and specific markers, demonstrating extensive reduction to practice, one skilled in the art would not require undue experimentation to identify the compounds having the desired biological function or to treat Alzheimer's disease.

Thus, where one skilled in the art routinely screens compounds for treating diseases and where compounds for treating diseases have been reduced to practice, having to do so is not the undue experimentation proscribed by 35 U.S.C. § 112, first paragraph, under the reasoning of *In re Wands*.

In *In re Angstadt*, 190 USPQ 214 (CCPA 1976), the court addressed the level of experimentation in an unpredictable art, *i.e.*, the chemical arts, where the claimed invention involved a method of catalytically producing hydroperoxides where the

specification admitted that not all disclosed complexes produced the hydroperoxides. The *Angstadt* Court, holding that the invention as claimed was enabled, reasoned:

> We note that many chemical processes, and catalytic processes particularly, are unpredictable. . . .
>
> Appellants have apparently not disclosed every catalyst which will work; they have apparently not disclosed every catalyst which will not work. The question, then, is whether in an unpredictable art, section 112 requires disclosure of a test with every species covered by a claim. To require such a complete disclosure would apparently necessitate a patent application or applications with "thousands" of examples or the disclosure of "thousands" of catalysts along with information as to whether each exhibits catalytic behavior resulting in the production of hydroperoxides. More importantly, such a requirement would force an inventor seeking adequate patent protection to carry out a prohibitive number of actual experiments. This would tend to discourage inventors from filing patent applications in an unpredictable area since the patent claims would have to be limited to those embodiments which are expressly disclosed. A potential infringer could readily avoid "literal" infringement of such claims by merely finding another analogous catalyst complex which could be used in "forming hydroperoxides."
>
> *In re Angstadt*, 190 USPQ at 218 (citations omitted).

Thus, where methods for assessing whether a claimed method of identifying a compound capable of treating Alzheimer's disease having the utility of the claimed methods and compounds are well-known in the art and/or disclosed in the specification, and where working examples are disclosed (see Figures 1-7 and Examples 1 and 2), it would not be undue experimentation to screen and identify compounds which have the disclosed utility where the art typically engages in such experimentation.

More recently, in *Ex parte Mark*, 12 USPQ2d 1904 (Bd. Pat. App. & Int. 1989), the Board reversed the Examiner's rejection for lack of enablement under 35 U.S.C. § 112, first paragraph, with regard to an application involving admittedly "innumerable" muteins comprising a non-essential cysteine which exhibit biological activity after modification to substitute the cysteine. In reversing the Examiner, the *Mark* Court stated:

> To the extent that the examiner is concerned that undue experimentation would be required to determine other proteins suitable for use in the present invention, we find [an applicant]'s declaration to be persuasive that only routine experimentation would be needed for one skilled in the art to practice the claimed invention for a given protein. The fact that a given protein may not be amenable for use in the present invention in that the cysteine residues are needed for the biological activity of the protein does not militate against a conclusion of enablement. One skilled in the art is clearly enabled to perform such work as needed to determine whether the cysteine residues of a given protein are needed for retention of biological activity.

Ex parte Mark, 12 USPQ2d at 1907. Therefore, where one skilled in the art routinely assays the compounds (*e.g.*, compounds capable of lowering isoprostane levels) for the asserted utility (*e.g.*, treating a disease), it is not undue experimentation for them to do so.

In sum, Applicants respectfully submit that there the claims are amply supported by the disclosure provided in the specification as filed, and that numerous working examples, which are not even required under the present law regarding enablement, are provided. Therefore, undue experimentation would not be required of a skilled artisan to make and/or use the full scope of the invention as recited in claims 24-34. Given the advanced state of the relevant art, the ample disclosure, and the extensive reduction to practice provided in the specification as filed, claims 24-34 are amply enabled and this requirement of 35 U.S.C. § 112, first paragraph, has been satisfied. Thus, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Rejection of Claims 1-34 pursuant to 35 U.S.C. § 112, second paragraph

Claims 1-34 stand rejected pursuant to 35 U.S.C. § 112, second paragraph as being indefinite. More specifically, the Examiner asserts that: in claim 1 "the level" lacks antecedent basis; in claim 13(c) it is unclear what is intended; in claim 20 there may be an inconsistency regarding step (a) which includes a second mammal; and in claim 25, "the optimal" lacks antecedent basis.

Applicants do not understand the Examiner's assertion that in claim 1 "the level" and in claim 25 "the optimal" lack antecedent basis. Applicants respectfully submit

that "the level" as used in claim 1 and "the optimal" as used in claim 25 do not require antecedent basis in the context in which they are used in the preamble. Applicants assert that "the level" in claim 1 and "the optimal" in claim 25 are used properly and request that the rejection be reconsidered and withdrawn.

Applicants, while not necessarily agreeing with the Examiner's reasoning, in a good faith effort to expedite prosecution of this application, have amended claim 13 to clarify what is intended. To that end, claim 13, parts (c) and (d) have been amended where the phrase "assaying said isoprostane marker from b)" as originally filed for part (c), has been deleted such that claim 13 now recites isolating the marker in part (b) as it did before, followed by "quantifying the level of said isoprostane molecular marker" in part (c), the latter phrase having been part (d). Thus, Applicants assert that claim 13 is now clear as to its intention, that is, following obtaining and isolating/purifying the marker in parts (a) and (b), the level of the isoprostane molecular marker for lipid peroxidation is quantified (now part c).

The amendment to claim 13 is fully supported throughout the specification as filed (see pages 10-40, Figures 1-7, Examples 1 and 2, and Claims 1-34) and no new matter had been introduced by way of this amendment.

Applicants respectfully submit that claim 20 is not inconsistent regarding step (a) which includes a second mammal. Claim 20, step (a), recites a method of measuring the level of an isoprostane marker in "either" a sample of a tissue or body fluid obtained from a first mammal prior to administering said compound, "or" from an otherwise identical second mammal which is not to be administered said compound. Thus, claim 20, step (a), contemplates two choices from which to obtain a sample to be used as a control. That is, the sample can be obtained from the animal which is to be treated with a compound, prior to treatment of that animal. Thus an animal serves as its own control. Or the control sample may be obtained from an otherwise identical second mammal, which has not and will not be treated with the compound. Thus, Applicants argue that claim 20, step (a), is not inconsistent regarding step a, which includes a second mammal, and in fact, Applicants assert that claim 20, step (a), merely provides alternative choices for deriving a control sample.

Applicants respectfully submit that claims 1, 13 (as amended), 20, and 25, are not indefinite under 35 U.S.C. § 112, second paragraph, nor are their dependent claims, thus Applicants request that the rejection of claims 1-34 be reconsidered and withdrawn.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been overcome or is now inapplicable, and that each of claims 1-5 and 8-34, claims 6 and 7 having been canceled herein, is in condition for allowance. Reconsideration and allowance of each of these claims are respectfully requested at the earliest possible date.

Respectfully submitted,
GARRET A. FITZGERALD *ET AL.*

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(Date)

KATHRYN DOYLE, Ph.D., J.D.
Registration No. 36,317
MORGAN, LEWIS & BOCKIUS, LLP
1701 Market Street
Philadelphia, PA 19103-2921
Telephone: (215) 963-5000
Direct Dial: (215) 963-4723
Facsimile: (215) 963-5299
E-Mail: kdoyle@morganlewis.com
Attorney for Applicants

KD/RLS/prw dlp
Enclosures (Petition for Two Month Extension of Time and fee therefor; Copy of IDS filed on February 1, 2002 and accompanying Form 1449)

Marked-up claims responsive to Office Action Dated February 28, 2002 (Paper No. 4)

1. (Amended) A method of measuring the level of lipid peroxidation in a mammal suspected of having an oxidant stress syndrome or disease, wherein said oxidant stress syndrome or disease is Alzheimer's disease, said method comprising

a) obtaining a first sample of a tissue or body fluid from said mammal;

b) assessing the level of an isoprostane molecular marker for lipid peroxidation present in said first sample, wherein said isoprostane molecular marker is selected from the group consisting of $iPF_{2\alpha}$-III, $iPF_{2\alpha}$-VI, and 8,12-*iso*-iPF_2-VI; and

c) comparing the level of said isoprostane molecular marker present in said first sample with the level of said isoprostane molecular marker present in a second sample of a tissue or body fluid obtained from an otherwise identical mammal which is not afflicted with an oxidant stress syndrome or disease, wherein an elevated level of said isoprostane molecular marker in said first sample relative to the level of said isoprostane molecular marker in said second sample, is indicative of an elevated level of lipid peroxidation in said mammal, thereby indicating the presence of an oxidant stress syndrome or disease in said mammal.

11. (Amended) A method of diagnosing an oxidant stress syndrome or disease in a mammal, wherein said oxidant stress syndrome or disease is Alzheimer's disease, said method comprising

a) obtaining a first sample of a tissue or body fluid from said mammal;

b) assessing the level of said isoprostane molecular marker present in said first sample, wherein said isoprostane molecular marker is selected from the group consisting of $iPF_{2\alpha}$-III, $iPF_{2\alpha}$-VI, and 8,12-*iso*-iPF_2-VI; and

c) comparing the level of said isoprostane molecular marker present in said first sample with the level of said isoprostane molecular marker present in a second sample of a tissue or body fluid obtained from an otherwise identical mammal which is not afflicted

with an oxidant stress syndrome or disease, wherein an elevated level of said isoprostane molecular marker in said first sample relative to the level of said isoprostane molecular marker in said second sample, is indicative of an elevated level of lipid peroxidation in said mammal, whereby said oxidant stress syndrome or disease is diagnosed in said mammal.

13. (Amended) A method of measuring the level of an isoprostane molecular marker for lipid peroxidation in a mammal suspected of having an oxidant stress syndrome or disease, said method comprising

a) obtaining a sample of a tissue or body fluid from said mammal;

b) isolating from said sample said isoprostane molecular marker by using a total lipids solvent extraction method; and

c) [assaying said isoprostane molecular marker from b); and

d)]quantifying the level of said isoprostane molecular marker.